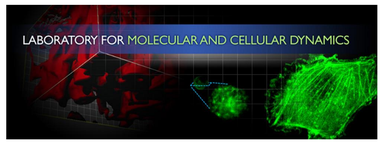
A simulated cytokine signaling network predicts that TAK1- and Smad7-mediated crosstalk facilitates interactions between TGF-β1- and TNFα-induced signaling in cancer cells



R. J. Seager[1], Fabian Spill[2], Ran Li[3], Roger D. Kamm[4], Muhammad H. Zaman[1,5]

[1]Boston University, Boston, MA [2]University of Birmingham, Birmingham, U.K. [3]Massachusetts General Hospital, Boston, MA
[4]Massachusetts Institute of Technology, Cambridge, MA [5]Howard Hughes Medical Institute, Boston, MA

BOSTON UNIVERSITY

Abstract

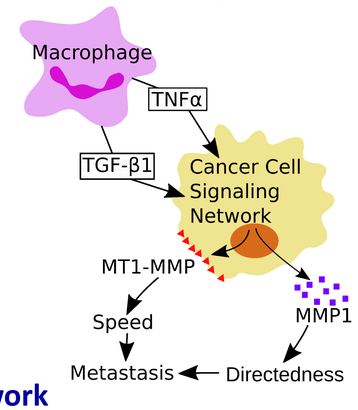
A tumor is not a homogenous mass of cancer cells, but is in fact a diverse microecosystem populated by many physical, chemical, and biological actors, all of which interact with each other and, together, drive gross tumor behavior. When small signaling molecules known as cytokines are expressed and secreted from a cell into the extracellular space, they can bind to corresponding receptors on the same cell or other cells, initiating intracellular signaling pathways capable of affecting many cell processes and behaviors. Two cytokines expressed and secreted by tumor-associated macrophages (TAMs), transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNFα), have been shown to modulate the speed and directedness of cancer cell migration, as mediated by changes in the extracellular matrix (ECM)-degrading enzymes membrane-type-1 matrix metalloproteinase (MT1-MMP/MMP14) and matrix metalloproteinase-1 (MMP1), respectively. These expression changes—and thus the migration effects—are driven by a nonlinear signaling network characterized by extensive crosstalk between the downstream intracellular signaling pathways activated by these cytokines, where migration directedness is controlled by a synergistic integration of TGF-β1 and TNFα activity and migration speed is more directly regulated by TGF-β1 activity alone.

In order to elucidate the intracellular signaling mechanisms and species responsible for these behaviors, we have constructed an ordinary differential equation signaling model describing the TGF-β and TNFα signaling pathways in cancer and how they interact. Limiting the model in scope to only the signaling pathways and motifs associated with TGF-β and TNFα signaling and MMP1 and MT1-MMP expression and informing the layout and parameters of this model from literature and experiments, we used this model to reproduce experimentally observed MMP expression patterns and explore the mechanisms underlying the observed synergistic interaction between the two pathways. Sensitivity analysis of this network revealed that TGF-β-activated kinase 1 (TAK1), an intermediate signaling protein indirectly activated by both TGF-β1 and TNFα, serves as an integrator of TGF-β and TNFα signaling, and Smad7, a transcriptionally-regulated signaling protein, serves as a mutually regulated inhibitor of both pathways, facilitating the observed signaling. Simulated perturbation of the network also showed that when the balance between these two important regulators is disrupted, the observed signaling behavior can no longer arise—predictions that were later validated experimentally.

By analyzing this system through mathematical modeling methods, we hope to gain a broader understanding of how interactions between tumor cells and their microenvironment affects their behavior and demonstrate the utility of these methods in cancer biology.

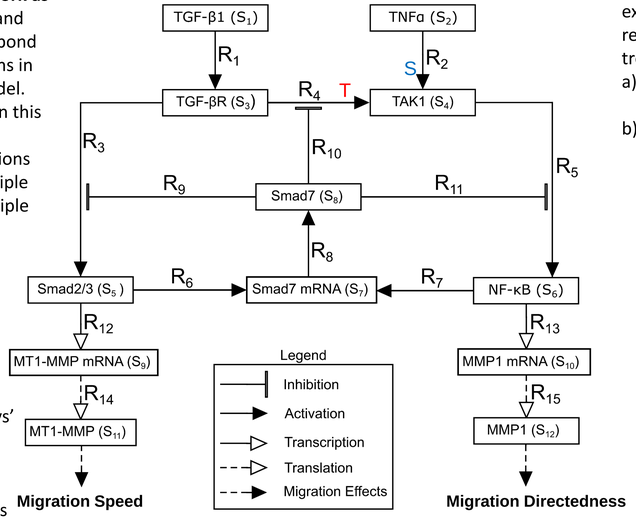
Prior Experimental Motivation

Fig. 1. Experimental data suggests that macrophage-secreted TGF-β1 and TNFα modulate cancer cell migration and directedness [1]. Crosstalk between the signaling pathways associated with these cytokines allows the emergence of unique phenomena such as the synergistically-induced heightened expression of MMP1 in the presence of both TGF-β1 and TNFα.



Hypothesized Signaling Network

Fig. 2. Proposed network as modeled. All species and network labels correspond to their representations in the mathematical model. Note that the edges on this network map may represent single reactions or composites of multiple reactions. TAK1's multiple phosphorylation sites (T = threonine site TAK phosphorylation, S = serine site TAK phosphorylation) allow it to act as a biological AND gate and integrate the two signaling pathways' signals, while Smad7 acts as a mutually regulated inhibitor. Their combined effects allow unique MMP signaling patterns to occur.



Modeling Framework

System of ODEs describing the concentrations of 12 signaling species over time:

- S_3, TGF-βR complex: $$\frac{dS_3}{dt} = \frac{p_1 S_1}{k_1 + S_1}\left(S_{3,tot} - S_3\right) - c_P S_3 \quad (1)$$
- $S_{4,S}$, TAK1 (Serine-Phos.): $$\frac{dS_{4,S}}{dt} = \left(\frac{p_2 S_2}{k_2 + S_2} + S_{2,0}\right)\left(S_{4,tot} - S_4 - S_{4,T} - S_{4,S}\right)\left(\frac{k_{10}}{k_{10} + S_8}\right) - c_P S_{4,T} \quad (2)$$
- $S_{4,T}$, TAK1 (Serine-Phos.): $$\frac{dS_{4,T}}{dt} = \left(p_4 S_3 + S_{4,0}\right)\left(S_{4,tot} - S_4 - S_{4,T} - S_{4,S}\right)\left(\frac{k_{10}}{k_{10} + S_8}\right) - c_P S_{4,S} \quad (3)$$
- S_4, TAK1 (Dual-Phos.): $$\frac{dS_4}{dt} = \left(\frac{p_2 S_2}{k_2 + S_2} + S_{2,0}\right) S_{4,T}\left(\frac{k_{10}}{k_{10} + S_8}\right) + \left(p_4 S_3 + S_{4,0}\right) S_{4,S}\left(\frac{k_{10}}{k_{10} + S_8}\right) - c_P S_4 \quad (4)$$
- S_5, Smad2/3: $$\frac{dS_5}{dt} = \left(p_3 S_3 + S_{3,0}\right)\left(S_{5,tot} - S_5\right)\left(\frac{k_9}{k_9 + S_8}\right) - c_P S_5 \quad (5)$$
- S_6, NF-κB: $$\frac{dS_6}{dt} = \left(p_5 S_4 + S_{5,0}\right)\left(S_{6,tot} - S_6\right)\left(\frac{k_{11}}{k_{11} + S_8}\right) - c_P S_6 \quad (6)$$
- S_7, Smad7 RNA: $$\frac{dS_7}{dt} = \frac{g_6 S_5}{k_6 + S_5} + \frac{g_7 S_6}{k_7 + S_6} - c_R S_7 \quad (7)$$
- S_8, Smad7: $$\frac{dS_8}{dt} = h_8 S_7 - c_P S_8 \quad (8)$$
- S_9, MT1-MMP RNA: $$\frac{dS_9}{dt} = \frac{g_{12} S_5}{k_{12} + S_5} - c_R S_9 \quad (9)$$
- S_{10}, MMP1 RNA: $$\frac{dS_{10}}{dt} = \frac{g_{13} S_6}{k_{13} + S_6} - c_R S_{10} \quad (10)$$
- S_{11}, MT1-MMP: $$\frac{dS_{11}}{dt} = h_{14} S_9 - c_P S_{11} \quad (11)$$
- S_{12}, MMP1: $$\frac{dS_{12}}{dt} = h_{15} S_{10} - c_P S_{12} \quad (12)$$

Parameter Definitions

p_i = activation/phosphorylation rate constant for reaction i
k_i = inhibitor or transcription factor binding constant for reaction i
$S_{i,0}$ = basal activation rate constant for reaction i
$S_{j,tot}$ = total amount of active and inactive species j in cell
c_j = dilution and degradation rate constant of mRNA (R) or protein (P)
g_i = transcription rate constant for reaction i
h_i = translation rate constant for reaction i

Implementation and Parameter Fitting

- The system of equations was implemented in Python and solved over a 48 hour period of simulated experimental time.
- 7 out of 32 total model parameters (b_1-b_5, k_9-k_{11}) were fit using gradient descent to minimize the squared error between the simulated results and the original experimental data.
- The remaining 25 parameters were derived from literature values.

Results

Fig. 3. Model reproduces fundamental trends in experimental data. Comparison between original experimental data and model results for different cytokine treatments:
a) steady state MMP1 concentration,
b) steady state MT1-MMP concentration.

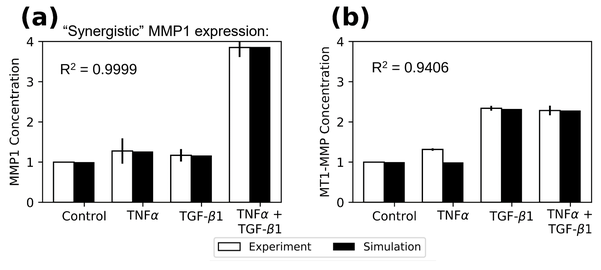
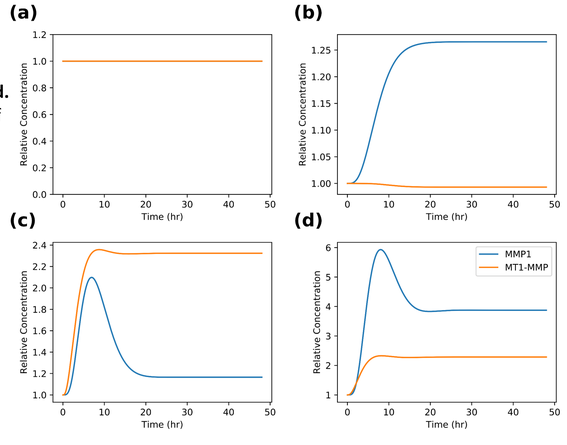
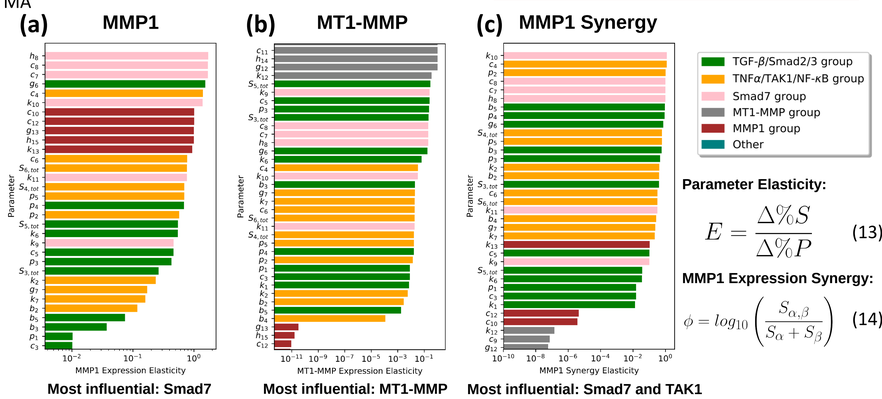


Fig. 4. Cytokine treatment induces transient behavior before steady state is achieved. Time-varying concentrations of output molecules MMP1 and MT1-MMP:
a) No cytokines
b) TNFα only,
c) TGF-β1 only,
d) both TNFα and TGF-β1.





Parameter Elasticity:

$$E = \frac{\Delta\%S}{\Delta\%P} \quad (13)$$

MMP1 Expression Synergy:

$$\phi = log_{10}\left(\frac{S_{\alpha,\beta}}{S_\alpha + S_\beta}\right) \quad (14)$$

Most influential: Smad7 **Most influential: MT1-MMP** **Most influential: Smad7 and TAK1**

Fig. 5. Certain reactions are stronger drivers of MMP expression than others. a-b) Parameter sensitivity studies giving elasticities for MMP1 and MT1-MMP expression when the parameter values are varied by 10% in both directions, respectively. The elasticity of the MMP expression is calculated according to Eq. 13 where S is the MMP expression level and P is the parameter value. c) MMP1 synergy sensitivity study to determine which parameters most control the synergistic induction of MMP expression by TGF-β1 and TNFα. The synergy is calculated according to Eq. 13 where $S = \phi$ is the MMP1 expression synergy calculated according to Eq. 14, where α and β subscripts indicate treatment with TGF-β1 or TNFα.

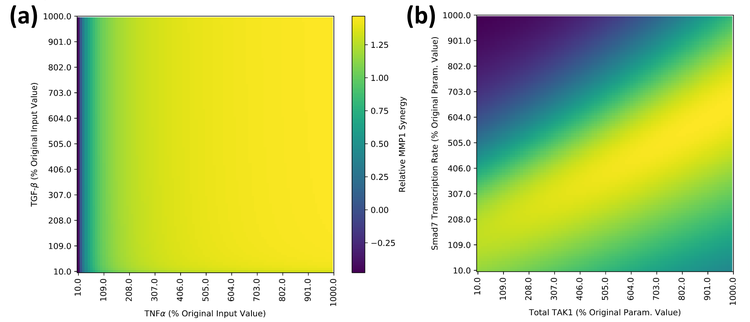
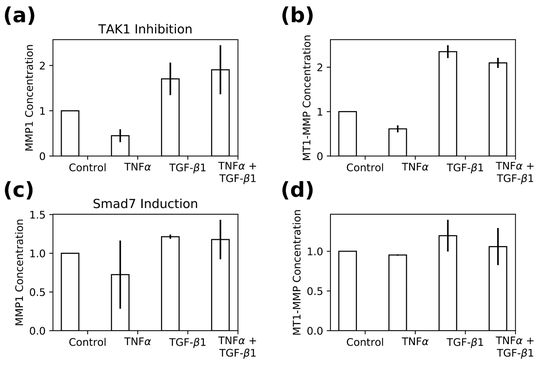


Fig. 6. The model predicts that changes in cytokine concentrations or the concentrations of key regulators TAK1 and Smad7 affect whether the synergistic MMP1 expression occurs. a) TNFα is a stronger driver of synergistic MMP1 expression than TGF-β1. b) Synergistic MMP1 expression is only possible when the concentrations of TAK1 and Smad7 have the right values relative to one another.

Experimental Validation

Fig. 7. Experimental perturbation of predicted key regulators TAK1 and Smad7 disrupts synergistic induction of MMP1 expression. a-b) When TAK1 activation is inhibited, the synergistic MMP1 expression is lost and MT1-MMP expression patterns change.
c-d) When the overexpression of Smad7 is induced, the synergistic MMP1 expression is lost and MT1-MMP expression patterns change.



Conclusions

- Our model recaptures the fundamental system behaviors, namely, the synergistic interactions between the TGF-β and TNFα signaling pathways underlying MMP1 expression.
- Our model supports the idea that TAK1 and Smad7 form the primary linkages between these two signaling pathways allowing for the observed synergy to occur.
- The observed synergistic MMP1 expression pattern requires a specific TAK1 and Smad7 levels, relative to each other, and in the absence of either TAK1 or Smad7, the synergistic behavior is lost.
- MMP expression and observed MMP1 synergy is heavily driven by a few important reactions, especially those related to the activities of TAK1 and Smad7.
- Subsequent experimental work perturbing TAK and Smad7 proved model predictions to be correct.

Acknowledgements

We gratefully acknowledge the support of NCI grant 5U01CA177799.

References

[1] Li, R. et al. Macrophage-Secreted TNFα and TGFβ1 Influence Migration Speed and Persistence of Cancer Cells in 3D Tissue Culture via Independent Pathways. Cancer Research 77, 279–290 (2017).
[2] Seager, R.J. et al. A simulated cytokine signaling network predicts that TAK1- and Smad7-mediated crosstalk facilitates interactions between TGF-β1- and TNFα-induced signaling in cancer cells. In preparation (2019).